Supporting Information

Transition state analysis of adenosine triphosphate phosphoribosyltransferase

Gert-Jan Moggré[a], Myles B. Poulin[b,d], Peter C. Tyler[c], Vern L. Schramm[d] and Emily J. Parker[a,c]*.

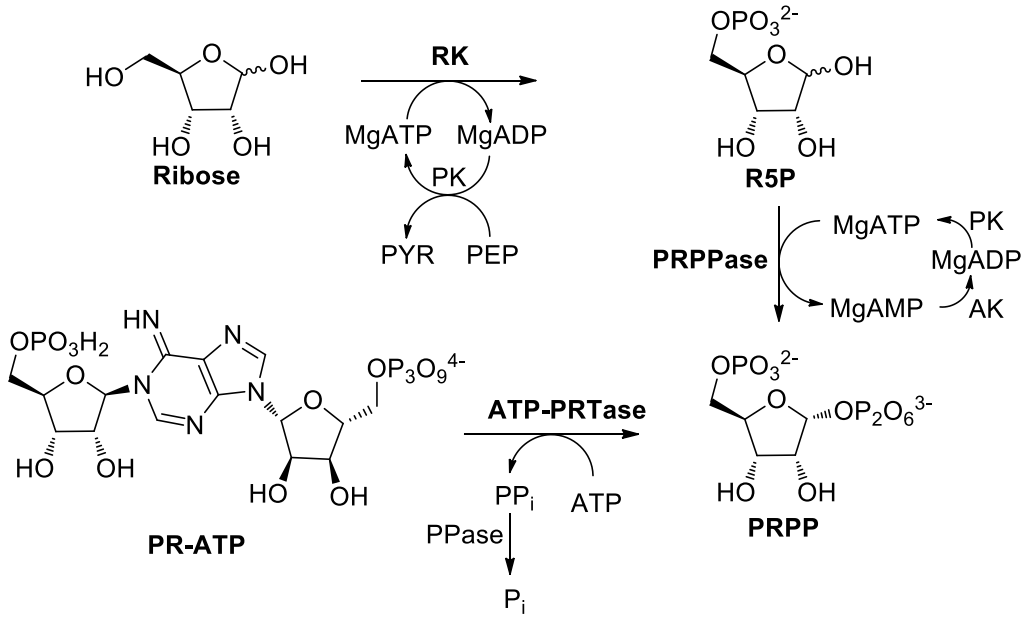
[a]Maurice Wilkins Centre, Biomolecular Interaction Centre and Department of Chemistry, University of Canterbury, PO Box 4800, Christchurch 8140, New Zealand. [b]Department of Chemistry and Biochemistry, University of Maryland College Park, College Park, Maryland 20742. [c]Ferrier Research Institute, Victoria University of Wellington, PO Box 33436, Petone 5046, New Zealand. [d]Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York 10461, United States.

Emily.parker@vuw.ac.nz

S1 Synthesis of labelled PRATP from ribose.

The [1′-^{3}H] and [1′-^{14}C]PRATP were synthesized from [1′-^{3}H] and [1′-^{14}C]D-ribose, respectively. The reaction mixture contained, 20-50 µCi of labelled substrate, 50 mM phosphate buffer (pH 8.5), 100mM NaCl, 10 mM $MgCl_2$, 20 mM phosphoenol pyruvate, 0.7 mM ATP, myokinase 5 units/mL, pyruvate kinase 20 units/mL, phosphoribosyl pyrophosphatese 32 µg/mL, *Cje*ATP-PRT 1.1 µM, *Eco*Pyrophosphatase 1.1 µM. Were [1′-^{3}H] D-ribose was used additional unlabelled ribose (0.5 mM) was added after 30 min incubation period. The reactions was initiated by ribokinase (40 µg/mL) and the mixture incubated at 25°C. The reaction progress was monitored by HPLC (Phenomenex kinetex, 5µ C18 100Å, 250x4.6mm, Buffer A; 50 mM KH_2PO_4 $(Bu)_4NHSO_4$ pH 5.0, Buffer B; 1:1 Buffer A:Acetonitrile). Reactions were left until no further reaction progress was observed or if the reaction time exceeded 2 hours, whichever came first as prolonged reaction times led to the formation of the Dimroth rearranged side product. Reactions were stopped by lowering the pH

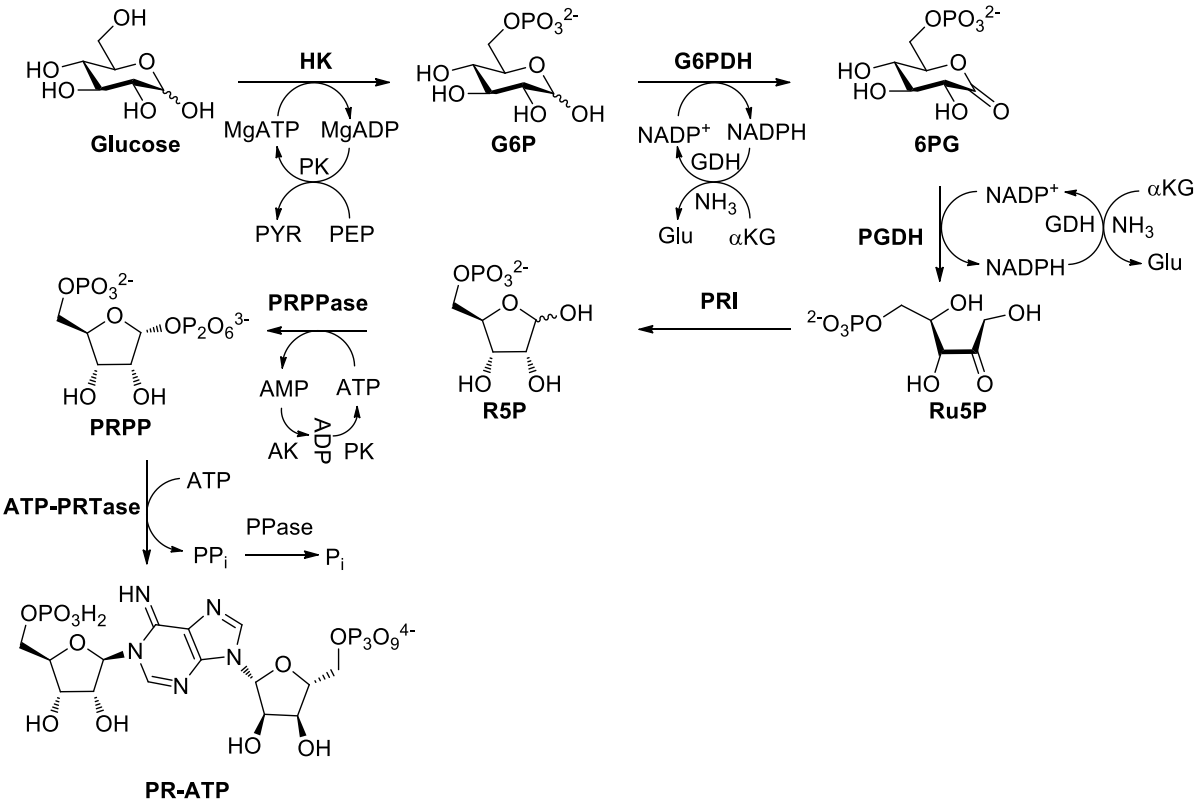
to 6.0 by the addition of 0.1M H_2SO_4. Lowering the pH also significantly reduced the formation of the rearranged side product. Purification of the isotopically labelled PRATP was then achieved according to the general procedure described below.



S2 Synthesis of labelled PRATP from D-glucose.

The [5′-^{3}H], [5′-^{14}C], [1-^{15}N,5′-^{14}C] and [6-^{15}N,5′-^{14}C]PRATP were synthesized from [6′-^{3}H] and [6′-^{14}C]D-glucose, respectively. The reaction mixture contained, 20-50 µCi of labelled D-glucose, 50 mM phosphate buffer (pH 8.5), 100 mM NaCl, 10 mM $MgCl_2$, 20 mM phosphoenol pyruvate, 10mM GlyGly, 400µM NADP, 20 mM α-Ketoglutarate, 6 mM NH_4Cl, 0.8 mM ATP, myokinase 5 units/mL, pyruvate kinase 20 units/mL, glucose 6-phosphate dehydrogenase 10 units/mL, phosphogluconate dehydrogenase 1 unit/mL, phosphoribose isomerase 2 units/mL, L-glutamate dehydrogenase 2 units/mL, phosphoribosyl pyrophosphatese 32 µg/mL, The reactions was initiated by the addition of hexokinase 1 unit/mL and incubated for 30 min after which *Cje*ATP-PRT (1.1 µM) and pyrophosphatase (1.1 µM) were added. Where [6′-^{3}H]glucose was used additional unlabelled glucose (0.5 mM) was added after 30 min and incubated another 30 min before the last two enzymes were included. The reaction progress

was monitored by HPLC (Phenomenex Kinetex, 5µ C18 100Å, 250x4.6mm, Buffer A; 50 mM KH_2PO_4 $(Bu)_4NHSO_4$ pH 5.0, Buffer B; 1:1 Buffer A:Acetonitrile) and the reactions were left until no further reaction progress was observed or if the reaction time exceeded 2 hours after the *Cje*ATP-PRT enzyme was added. Reactions were stopped by lowering the pH to 6.0 with 0.1M H_2SO_4. Purification of the isotopically labelled PRATP was then achieved according to the general procedure described below.

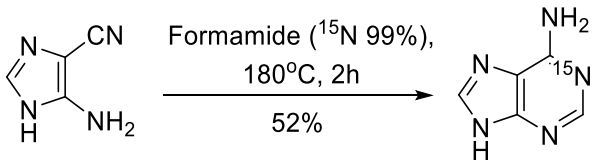


General procedure for the purification of PRATP.

- Isotopically labelled PRATP was purified by HPLC (Phenomenex kinetex, 5µ C18 100Å, 250x4.6mm, Buffer A; 50 mM KH_2PO_4 $(Bu)_4NHSO_4$ pH 5.0, Buffer B; 1:1 Buffer A:Acetonitrile, 0-2 Min 100% Buffer A, 2-25 Min 0-100% Buffer B, 25-27 Min 100% Buffer B, 27-28 Min 100-0% Buffer B with a constant flow of 1mL/Min), followed by

drying using speed vacuum evaporation. Samples were desalted shortly before KIE measurements were made using HPLC (Phenomenex kinetex, 5µ C18 100Å, 250x4.6mm, Buffer A; 50mM $AcONH_4$ pH 6.0, Buffer B; 1:1 Buffer A:Acetonitrile, 0-5 Min 100% Buffer A, 5-20 0-100% Buffer B and 20-25 Min 100% buffer B with a constant flow of 1mL/Min). To prevent degradation at this point, the samples were dried for no longer than 10-15 min in small volumes (<200 µL) by speed vacuum evaporation, immediately after collection. After concentration the product was taken up in a small amount of water, divided into small aliquots, the radioactivity level was tested, and then the samples were frozen at -80 °C until further use. Each aliquot was used once and any remaining label was re-purified before further use.

S3 Synthesis of [1-^{15}N]adenine



3.5 **3.6**

[1-^{15}N]adenine: A mass spectrometry vial (2 mL) containing a stir bar and 4-amino-imidazole-5-carbonitrile (0.49 mmol, 53 mg) was flushed with a gentle flow of nitrogen gas for 30 min. 0.5 mL of ^{15}N formamide (Cambridge Isotope Laboratories) was added and the 4-amino-imidazole-5-carbonitrile was fully dissolved before heating on a preheated oil bath at 180 °C for 2.5 hours with active stirring. The reaction was allowed to cool down upon which solids formed. The crude reaction mixture was further cooled on ice for 30 min and solids filtered over a cotton wool plug. The excess ^{15}N formamide was recovered. The solids were washed 2 times with ice cold water to give the final [1-^{15}N]adenine product as a yellow powder which was used without further purification. (35 mg, 52% yield). The spectroscopic analysis was in agreement with reported literature for this structure.[1] IR (ν): 537.6, 633.1, 720.3, 793.6, 843.1,

903.5, 936.3, 1123.3, 1246.2, 1305.0, 1365.7, 1416.3, 1448.9, 1591.1, 1668.5, 2788.4, 3107.4 cm^{-1}): ^{1}H NMR (400 MHz, DMSO-D6); δ 7.06 (bs, 2H, NH_2), 8.07 (s, 1H, H8), 8.09 (d, *J* = 15.3 1H, H2); HRMS(ESI) *m*/*z* calcld. for $[C_5H_6N_4{}^{15}N]^+$: 137.0588 obsd.: 137.0583.

S4 Measurement of kinetic isotope effects

The KIEs were measured under competitive conditions using dual channel liquid scintillation counting.[18] An experiment included four reactions that were quenched at partial conversion (20%-40%), two 100% conversion reactions and a negative control containing all components except for the enzyme to account for non-enzymatic degradation. A master mix containing all components except the enzyme was prepared containing equal counts of ^{3}H and ^{14}C labeled PRATP, with one label at a reaction sensitive position, for example [1′-^{3}H]PRATP, and the appropriate remote labeled substrate, in this case [5′-^{14}C]PRATP. The master mix contained at least 315,000 cpm/per isotope (45,000 cpm/per isotope/reaction). Additional unlabeled PRATP was added to give a final chemical concentration of PRATP of 10 μM for reactions with *Mtu*ATP-PRT, 19 μM for reactions with *Cje*ATP-PRT, and 35 μM for *Lla*ATP-PRT reactions. Reaction mixtures were prepared in Tris buffer (100 mM NaCl, 50 mM KCl, 10 mM $MgCl_2$ and 50 mM Tris HCl pH 8.5) and phosphonoacetic acid 10 mM. The master mix was divided into seven equal portions and an appropriate amount of enzyme and/or buffer was added to give a final reaction volume of 200 μL. Enzymes were added to a final concentration of 240 nM or 16 μM for *Mtu*ATP-PRT, 1.7 μM or 18 μM for *Cje*ATP-PRT, and 150 nM or 940 nM for the *Lla*ATP-PRT enzyme to achieve partial (20-40%) or full (100%) conversion. Reactions were carried out at 25˚C for 10-20 min followed by acidification with 0.1 M H_2SO_4 to pH 6.0. Reactions were processed immediately after completion by loading the reaction mixture onto a pre-equilibrated Grace Extract-Clean SPE carbo 150 mg/4 mL column (pre-equilibrated with 4 mL water and 4 mL elution buffer (50 mM $(NH_4)_2CO_3$ at pH 6.0 with conc. AcOH)). Columns

were eluted with 4x 1 mL of elution buffer and the flowthrough was collected directly into a scintillation vial. Samples were dried under speed vacuum evaporation for at least 12 hours and the resulting solids were taken up in MilliQ water (500 μL). 10 mL Ultima Gold™ scintillation fluid (Perkin Elmer) was added, samples were mixed for 2x 20 sec on a vortex mixer and left to stand for 1 hour to allow the formed air bubbles to clear. Each vial was then counted for 10 cycles of 10 min in dual channel format. Channel A was set for ^{3}H (0-25 keV) and channel B for the remaining ^{14}C signal (25-400 keV). In each sample set, a ^{14}C standard was included, which was treated the same as the reaction samples, to correct for channel overlap between the two isotopes. The KIEs were determined using Equation 1.[23] KIE values measured using the [5´-^{3}H]PRATP remote label were corrected for the remote isotope effect according to Equation 2. KIEs for each position were determined from at least two independent experiments with a minimum of eight replicates in total. Reliability of the results is reported as standard error of the mean (SEM).

$$(KIE_{obs}) = \frac{\log(1-f)}{\log(1-f\,R_p/R_0)} \qquad \text{(Equation S1)}$$

$$KIE_{corrected} = KIE_{obs} * KIE_{Remote} \qquad \text{(Equation S2)}$$

S5 Forward commitment to catalysis by isotope trapping

The forward commitment to catalysis, or the partitioning of the Michaelis complex to product relative to substrate release (k_{cat}/k_{off}), was measured by isotope trapping.[26] ATP-PRT (20 μM, final concentration) was incubated with [5′-^{14}C]PRATP (50 μM, 140,000 cpm final

concentration) in (100 mM NaCl, 50 mM KCl, 10 mM $MgCl_2$ and 50 mM Tris HCl pH 8.5) to give a total volume of 20 μL. A chase solution containing 500 μM unlabeled PRATP and 10 mM PA was rapidly added (480 µL) to the 20 μL. 120 µL samples were then taken after 1, 2, 4, and 8 catalytic turnovers and quenched onto 10 μL H_2SO_4 0.1 M and quantified for product formation (d-α-5-phosphoribosyl-1-phosphonoacetic acid (PRPA)) according to the above described SPE charcoal method. A no enzyme control was added to correct for non-enzymatic degradation. Two replicates of this experiment were done. The concentration of PRPA formed after the addition of the chase solution as a function of time was plotted. The fraction of PRPA product formation versus bound PRATP following dilution in excess unlabeled PRATP can then be found by extrapolating back to zero time.

$$[EA] = \frac{[E_t][^{14}\text{C-PRATP}]}{K_\text{m} + [^{14}\text{C-PRATP}]} \qquad \text{(Equation S3)}$$

$$Y = \frac{[\text{PRPA}]}{[EA]} \qquad \text{(Equation S4)}$$

$$C_f = \frac{Y}{1 - Y} \qquad \text{(Equation S5)}$$

$$KIE = KIE_{exp}(1 - C_f) - C_f \qquad \text{(Equation S6)}$$

S6 Crystal parameters, data collection and refinement statistics for ATP bound *Mtu*ATP-PRT crystal structure

	ATP
Data collection	
Crystal system, space group	Trigonal, $H3_2$
Unit cell parameters	
a, *b*, *c* (Å)	134.76, 134.76, 111.00
α, β, γ (°)	90, 90, 120
Resolution range (Å)	40.99-2.40

Measurements	303991
Unique reflections	15289
Redundancy	20.0 (20.0)
Completeness (%)	100 (100)
$I/\sigma(I)$	20.0 (2.4)
R_{mean}	0.143 (1.595)
$CC_{1/2}$	0.999 (0.773)
Wilson *B*-value ($Å^2$)	49.6
Mathews coefficient	3.16
Refinement	
Resolution (Å)	38.9-2.40
R_{cryst}	0.216
R_{free}	0.250
Chain length	287
Observed number of residues	281
Water molecules	30
Other	6
Mean *B* ($Å^2$)	
Protein	36
Water	45
Other (SO_4^{3-}, Mg^{2+})	58
Ligand (ATP)	41
RMSD from target values	
Bond lengths (Å)	0.01
Bond angles (°)	1.5
Dihedral angles (°)	0.08
Ramachandran	
Preferred (%)	98
Allowed (%)	2
Outliers (%)	0
PDB Code	5U99

S7 Coordinates for the ground state structure

O	1.74502200	-1.85686900	1.18272500
O	3.03877600	-3.15982500	-0.68110200
O	3.97982900	-0.89795000	0.25482700
P	2.77551700	-1.82832600	0.01124000
C	2.30421000	0.23014800	-1.80901700
O	1.78321400	-0.88495500	-1.15478300

C	1.87397900	1.56433400	-1.17199800
O	0.41440300	1.70160100	-1.16503600
C	2.27917000	1.71760700	0.28858100
O	2.34264400	3.12176500	0.63927300
C	1.10773600	1.03891400	1.00427000
O	0.93751500	1.50816800	2.35872700
C	-0.08819100	1.44253500	0.13513300
N	-1.23045400	0.49861300	0.07352400
C	-1.07678900	-0.83177100	0.40666400
N	-2.06884700	-1.69040500	0.50625800
C	-3.27675100	-1.14073200	0.23978900
C	-3.57299300	0.15220000	-0.16264700
C	-2.47675700	1.07626500	-0.37147500
N	-2.61676700	2.24779200	-0.88987900
N	-4.93600300	0.30456400	-0.36240700
C	-5.44931000	-0.87131200	-0.07734200
N	-4.49030500	-1.79279000	0.29488200
H	3.40653400	0.21789000	-1.81876300
H	1.94088900	0.24127400	-2.85367700
H	2.25777000	2.40175300	-1.77088600
H	3.22149000	1.20174400	0.50313900
H	2.10391800	3.14866400	1.57936200
H	1.30092500	-0.04317200	1.02694600
H	-0.52124400	2.36479700	0.54611000
H	-0.06043600	-1.20049500	0.60878600
H	-1.71522400	2.69450200	-1.04967500
H	-6.50111900	-1.12294800	-0.11393000
H	-4.61202100	-2.76483800	0.53505100
H	1.26534900	0.78670800	2.91349100

S8 Coordinates for the *Mtu*ATP-PRT transition state structure

Mg	-2.21702500	-0.93467800	0.64720600
P	-1.95099000	1.90296500	-0.03929100
C	0.32669600	-0.90375800	-2.05065300
O	-1.23881500	0.53432000	-0.30605900
O	-2.02274400	2.21624600	1.45011100
O	-3.98444200	-0.36060700	0.07374400
C	-0.10106400	-2.13965300	-1.30291600
O	-1.49136700	-2.17977000	-1.13556200
O	-1.08512800	3.01866800	-0.84417800
C	0.71492600	-2.03265000	0.03675800
O	-0.22600500	-1.79309500	1.06985100
C	1.71735600	-0.88822600	-0.22119300
O	1.34001000	-0.33755000	-1.57934400
O	-3.43714700	-2.67443700	0.88657200
O	-1.88774700	-0.09447100	2.52407600
H	-0.09421600	-0.52362900	-2.98094100

H	0.25633200	-2.99202000	-1.90248800
H	1.24485400	-2.96945800	0.24144400
H	1.56805700	-0.02054900	0.42599000
H	-4.21211900	-2.12363700	0.60575200
H	-1.92075100	0.90438600	2.26169600
H	-1.84150800	-3.08500000	-1.12678400
H	-3.70819900	-3.24639400	1.61576000
H	-2.46905100	-0.20303100	3.28823300
C	-3.58347600	1.86843800	-0.84737800
H	-3.42245800	1.83207500	-1.93151200
H	-4.10210400	2.80908300	-0.64123900
C	-4.53576200	0.69783400	-0.47457600
O	-5.71887800	0.80859400	-0.73793600
H	0.14411900	-1.33752200	1.84343600
H	-0.98100700	3.84026000	-0.34088000
C	3.18487900	-1.29342100	-0.27276600
H	3.49298400	-1.56760400	0.74295000
H	3.33908100	-2.15475500	-0.93260600
O	3.94773100	-0.21179000	-0.78608900
P	4.94686300	0.67076100	0.14442900
O	3.90851800	1.02583900	1.33216000
H	4.25117600	1.65604000	1.98524500
O	5.90161800	-0.41805500	0.85864300
H	6.81200100	-0.38675800	0.52389900
O	5.61535200	1.75703000	-0.58413800

S9 Coordinates for the *Cje*ATP-PRT transitions state structure

Mg	-2.22516700	-0.98172300	0.62075600
P	-2.06786400	1.89143200	-0.00273200
C	0.32295500	-0.65955000	-1.99634200
O	-1.30665300	0.55161700	-0.29341700
O	-2.14308800	2.17184000	1.49174700
O	-4.01326700	-0.45020900	0.07187000
C	-0.05813700	-1.97539300	-1.36901800
O	-1.44484200	-2.10031700	-1.21444100
O	-1.24528100	3.04886400	-0.79347600
C	0.74845800	-1.95657200	-0.02200200
O	-0.19542700	-1.76553200	1.02015900
C	1.76373500	-0.81186700	-0.21447500
O	1.34698500	-0.13184000	-1.49692500
O	-3.37488600	-2.76087600	0.83565400
O	-1.90579600	-0.16203000	2.51256500
H	-0.11873800	-0.20072000	-2.87939300
H	0.34188000	-2.75130300	-2.04146600
H	1.26619800	-2.90918000	0.13460000
H	1.66749400	-0.00704000	0.51832900
H	-4.17964700	-2.24431700	0.57892800

H	-1.98224400	0.83874600	2.27717500
H	-1.74778600	-3.01813500	-1.29835200
H	-3.60690600	-3.37285100	1.54538800
H	-2.46750600	-0.31297900	3.28420000
C	-3.69928100	1.80960100	-0.80738800
H	-3.54024500	1.79537200	-1.89237400
H	-4.25085700	2.72763900	-0.58552000
C	-4.60650800	0.59874100	-0.44881300
O	-5.79527400	0.67446600	-0.69890700
H	0.15033400	-1.27639100	1.78458900
H	-1.14839800	3.85868800	-0.26994900
C	3.21870600	-1.24152700	-0.36059700
H	3.55881700	-1.61440000	0.61235300
H	3.32670700	-2.04152600	-1.10181800
O	3.98743600	-0.13358100	-0.80468700
P	5.04168400	0.63975700	0.16100300
O	4.05517800	0.91449300	1.41298800
H	4.43953500	1.47324800	2.10665700
O	5.99099200	-0.52961000	0.74288600
H	6.89009900	-0.49018800	0.37971800
O	5.71347300	1.76991200	-0.49406500

S10 Coordinates for the *Lla*ATP-PRT transitions state structure

Mg	-2.23755700	-1.03361100	0.58973700
P	-2.11840600	1.87546000	0.03927400
C	0.28473100	-0.41947200	-1.91359300
O	-1.34217400	0.54526500	-0.27882700
O	-2.18798000	2.11995300	1.53803900
O	-4.03060300	-0.49388800	0.06711800
C	-0.04696000	-1.81029300	-1.43391200
O	-1.42657700	-2.01589600	-1.29655800
O	-1.30673400	3.05034400	-0.73613700
C	0.75826000	-1.90187400	-0.09294400
O	-0.18391500	-1.77857600	0.96368300
C	1.78215400	-0.75561900	-0.20713800
O	1.32813800	0.05654600	-1.39178300
O	-3.36012400	-2.82220600	0.76661500
O	-1.92510700	-0.24903100	2.50162300
H	-0.17162000	0.11754100	-2.74194300
H	0.38565200	-2.49200900	-2.18357900
H	1.26613000	-2.86829100	-0.00460000
H	1.73491800	-0.03394900	0.61202300
H	-4.18151400	-2.32384100	0.53366900
H	-2.01262600	0.75347800	2.29858200
H	-1.68492300	-2.93223800	-1.48045200
H	-3.56768800	-3.47782400	1.44398400
H	-2.48247200	-0.42970600	3.27013200

C	-3.74917200	1.78858300	-0.76357700
H	-3.59339200	1.79859300	-1.84907900
H	-4.31088300	2.69516900	-0.52089300
C	-4.63946400	0.55844400	-0.42558400
O	-5.83049500	0.62744600	-0.66658800
H	0.14982900	-1.29647800	1.73767100
H	-1.19424300	3.84613200	-0.19433400
C	3.22258200	-1.19054300	-0.45464600
H	3.59510700	-1.66646600	0.45943500
H	3.28616400	-1.91113400	-1.27791900
O	3.99460300	-0.05463400	-0.81572100
P	5.09969500	0.60140100	0.17816400
O	4.16812100	0.76856800	1.49020300
H	4.59455600	1.24319700	2.22094500
O	6.04683700	-0.63480600	0.60589300
H	6.93237600	-0.57405600	0.21354100
O	5.77016000	1.77928400	-0.38846600